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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/637,216	08/11/2000	Scott J Hultgren	WSHU2005.1	7884
321	7590	01/13/2006	EXAMINER	
SENNIGER POWERS ONE METROPOLITAN SQUARE 16TH FLOOR ST LOUIS, MO 63102			ZHOU, SHUBO	
			ART UNIT	PAPER NUMBER
			1631	

DATE MAILED: 01/13/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Supplemental
Notice of Allowability

	Application No.	Applicant(s)
	09/637,216	HULTGREN ET AL.
	Examiner Shubo (Joe) Zhou	Art Unit 1631

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. This communication is responsive to _____.
2. The allowed claim(s) is/are 1,4,5,8,9,13,14,16,17,19,136-139,159 and 160.
3. Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 - a) All
 - b) Some* c) None
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
5. CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 - (a) including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
 - 1) hereto or 2) to Paper No./Mail Date _____.
 - (b) including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. Notice of References Cited (PTO-892)
2. Notice of Draftsperson's Patent Drawing Review (PTO-948)
3. Information Disclosure Statements (PTO-1449 or PTO/SB/08),
Paper No./Mail Date _____
4. Examiner's Comment Regarding Requirement for Deposit
of Biological Material
5. Notice of Informal Patent Application (PTO-152)
6. Interview Summary (PTO-413),
Paper No./Mail Date _____
7. Examiner's Amendment/Comment
8. Examiner's Statement of Reasons for Allowance
9. Other _____.

Examiner's Amendment

1. This supplemental action is to amend the specification to include missing parts on pages 72 and 78, respectively, by Examiner's Amendment.

2. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Timothy McBride.

In the specification:

on page 72:

line 1, before "(Fig. 6E).", insert the following

-- A complete data set to a resolution of 2.7 Å was collected in the laboratory setting (Rigaku Raxis IV image plate mounted on a Rigaku RU200 rotating anode X-ray generator) using an oscillation range of 1.5° and exposure time of 45 mm/frame ("Native" data set in Table 4). Se-Met PapD-PapK co-crystals were in the same space group with the same cell dimensions. Once cooled, these co-crystals diffracted to slightly higher resolution in the laboratory setting and a complete data set ("Se-Met Single" in Table 4) to a resolution of 2.5 Å was collected (2.5E oscillation range, 60 mm/frame). These co-crystals were also used to collect MAD data at the National Synchrotron Light Source at Brookhaven National Laboratory (Beamline X4A). Complete data sets at four wavelengths to a resolution of 2.4 Å were collected ("Se-Met1-4" in Table 4). All data were reduced and processed using the programs DENZO and SCALEPACK [Z. Otwinoski, in *Proceedings of the CCP4 Study Weekend*, L. Sawyers, N. Isaacs, S. Bailey, Eds. (SERC Daresbury Laboratory, Warrington, 1993), pp. 56-62].

Structure of PapD-PapK co-complex. The structure of the PapD-PapK co-complex was solved using MAD phasing [W. A. Hendrickson, *Science* 254, 51 (1991)]. The PapD-PapK co-complex contains three methionines, all of which

are in PapD, at positions 18, 66, and 172. The "Native" and "Se-Met Single" data sets were first used to generate a difference Patterson map using the program HEAVY [T. C. Terwilliger and D. Eisenberg, *Acta Crystallogr. A39*, 813 (1983)] where strong peaks could be readily located. Three heavy metal positions were determined using the program HASSP [T. C. Terwilliger, S.-H. Kim, D. Eisenberg, *Acta Crystallogr. A43*, 1 (1987)]. Initial SIRAS-solvent flattened phases were, however, insufficient to build a model of PapK. Subsequently, multi-wavelength anomalous diffraction (MAD) data were collected (Table 4). After local scaling using the high energy remote wavelength ("SeMet-4" in Table 4) as the reference wavelength, MAD phases were calculated using SHARP [E. De La Fortelle and G. Bricogne, *Methods Enzymol.* 276, 472 (1997)]. An interpretable electron density map was readily obtained after density modification by solvent flipping (program SOLOMON [J. P. Abrahams and A. G. W. Leslie, *Acta Crystallogr. D52*, 32 (1996)]). The PapD subunit was rebuilt into the experimental electron density, starting from the apo-PapD structure. A C_V trace of the PapK subunit was built into the experimental electron density map using program O [T. A. Jones and S. Thirup, *EMBO J.* 5, 819 (1986); T. A. Jones, J. Y. Zou, S. W. Cowan, M. Kjeldgaard, *Acta Crystallogr. A47*, 110 (1991)], accounting for all but 8 residues located at the NH₂-terminus, for which, even at later stages of the refinement, no electron density was observed. The electron density was of sufficient quality (Fig. 1) to unequivocally assign the sequence. The model was then refined using CNSsolve 0.5 [A. T. Brünger et al., *Acta Crystallogr. D54*, 905 (1998)] against the 'SeMet-3' structure factor amplitudes using the maximum likelihood refinement target with incorporation of experimental phase information [P. D. Adams, N. S. Pannu, R. J. Read, A. T. Brünger, *Proc. Natl. Acad. Sci.* 94, 5018 (1997); N. S. Pannu, G. N. Murshudov, E. J. Dodson, R. J. Read, *Acta Crystallogr. D54*, 1258 (1998)]. Both positional and simulated annealing refinement in cartesian space were used (the temperature factors were set to 25 Å²) and resulted in values of R- and free-R of 27.4 and 32.5 %, respectively [A. T. Brünger, *J. Mol Biol.* 203, 803 (1988)]. After two rounds of rebuilding, where simulated annealing omit maps were generated for ambiguous regions and used to adjust the model [A. Hodel, S.-H. Kim, A. T. Brünger, *Acta Crystallogr. A48*, 851 (1992)], positional refinement followed by restrained refinement of the temperature factors resulted in a model with R and free-R values of 24.3 and 28.8%, respectively. At this stage, 104 well-defined water molecules were added resulting in a final model with R- and free-R values of 23.8% and 27.4%, respectively. The stereochemistry of the model is excellent and the temperature factors restrained appropriately (Table 4). The model of PapK is complete between residues 9 and 157. Electron density was poor for residues 216 to 218 of PapD and therefore, this region was not included in the final model. Also, for the same reason, residues Arg⁹⁶ and Glu⁹⁸ in PapD were built as alanines. All residues in PapK and PapD are located in either the most favored or the allowed regions of the Ramachandran plot [G. N. Ramachandran

and V. Sasisekharan, *Adv. Protein Chem.* 23, 283 (1968)]. Coordinates have been deposited at the Protein Data Bank (entry code 1PDK).

COOH-terminally truncated Ig fold of PapK. PapK has the same overall variable-region immunoglobulin-like (Ig) fold as the amino-terminal domain of PapD, with two beta-sheets coming together in a beta-sandwich (Figs. 2A and 3A; see also Fig. 2A for secondary structure notation). However, the Ig fold of PapK is incomplete: it lacks the COOH-terminal seventh strand, G, which in canonical Ig folds forms an antiparallel beta-sheet interaction with strand F and contributes to the hydrophobic core of the protein. Remarkably, in the PapD-PapK co-complex, this missing strand is provided by PapD, which donates its G₁ beta-strand to complete the Ig fold of PapK (Figs. 2A, 2B, and 3A). The Ig fold thus produced is however atypical, since the donated strand runs parallel, rather than antiparallel, to strand F in PapK. The insertion of the G₁ beta-strand into the fold of the pilin, coined as “donor strand complementation” has important implications for the mechanisms of subunit folding, capping and assembly.

The first eight NH₂-terminal residues of PapK are disordered. The Ig fold of PapK (Fig. 3A) begins with a short beta-strand, A1, which makes typical antiparallel hydrogen bonds with the COOH-terminal residues of strand B. This short beta-sheet arrangement is interrupted by the insertion of a 3₁₀ helical turn (Figs. 2A and 3B) which results in strand A switching sides in the beta-sandwich in order to make antiparallel beta-strand interactions with the G₁ beta-strand of the chaperone (Fig. 3A). Strands A and B are connected by a short α -helix (α B in Figs. 2A and 3B) which precedes three successive aromatic residues (Phe³⁵, Trp³⁶, Tyr³⁷, Fig. 3B). While Phe³⁵ inserts into the hydrophobic core of the beta-sandwich, Trp³⁶ and Tyr³⁷ interact closely with residues at the COOH-terminus of helix α D (Fig. 2A), possibly contributing to its stability. Strand B forms the edge of one of the two beta-sheets in the beta-sandwich and runs antiparallel to strand E. Following strand B, the structure crosses over to the other side of the beta-sandwich through a short 3₁₀ helix (Fig. 2A) to form strand C1, which runs antiparallel to strand F. The COOH-terminus of strand C1 deviates from the beta-sheet arrangement to form a protruding beta-meander (strands C' and C''). Strand C'' reaches over to the other side of the beta-sandwich to form main-chain hydrogen bonds with strand D1. This small beta-structure eventually returns, as C2, to make main-chain hydrogen bonding interactions with strand F (Figs. 2A, 3A, and 3B).

An extended loop links strand C to strand D1 on the other side of the beta-sandwich. Strand D constitutes an edge of the D, E, B, A1 beta-sheet. It therefore runs antiparallel to strand E. However, strand D is divided in the middle by an insertion which meanders towards the C', C'' meander and reaches back to the E strand. Strand E is followed by a three-turn helix (α D) and a long loop structure which connects it to the COOH-terminal strand F. Finally, strand F, from Asp¹⁴⁵ onward, forms a parallel beta-sheet with strand G₁ of PapD (Figs. 2A and 3A). Hence, strand G₁ of PapD is an integral part of the C, F, A2 beta-sheet of PapK.

Structure of PapD in the PapD-PapK Co-complex. Except for the F₁-G₁ loop in the NH₂-terminal domain (Figs. 3C and 4), the structure of PapD in the PapD-PapK co-complex superimposes very well with apo PapD (r.m.s. deviation in C α atom positions, excluding the F₁-G₁ loop, of 0.65 Å). Hence, the binding of PapK does not alter the orientation of the domains of PapD. The major difference between the apo and PapK-bound forms of PapD is a large conformational change in the F₁-G₁ loop of PapD. The tip of this loop undergoes a flap motion of about 11 Å that results in an re-ordering of the F₁-G₁ loop such that residues 101 to 105 of PapD become part of the G₁ beta-strand.

The PapD-PapK interface. The total buried surface area in the PapD-PapK co-complex is 3434 Å². There are two distinct sites on PapK that interact with two corresponding sites on PapD. Site K1 of PapK interacts with a site on the NH₂-terminal domain (domain 1) of PapD (site D1) and site K2 of PapK interacts with a site on the COOH-terminal domain (domain 2) of PapD (site D2) (Fig. 5).

Site K1 contains a deep groove which runs the length of the subunit. The edges of the groove consist of strands A and F and its base is formed by the hydrophobic core of PapK (Figs. 6A, 6B and 6E). This groove is the result of the missing G beta-strand in the Ig fold of PapK. Site D1 includes residues 101 to 112 of the G₁ beta-strand of PapD, which insert into the K1 groove and make a beta-zipper interaction with strand F of PapK on one side of the groove. Residues 101 to 105 also make a beta-zipper interaction with strand A2 on the other side of the groove (Figs. 6A and 6B). Insertion of the G₁ beta-strand also results in the formation of a continuous 5-stranded beta-sheet which includes strands C₁, F₁, and G₁ of PapD and F and C₁ of PapK (Fig. 2A). The alternating hydrophobic residues in the G₁ beta-strand of PapD (Leu¹⁰³, Ile¹⁰⁵, and Leu¹⁰⁷) interact with the hydrophobic base of the groove --

on page 78,

line 1, before “with the G1 strand ...”, insert the following

-- Example 4: FimC-FimH Co-complex Structure

In the FimC-FimH co-complex, the seventh strand (G₁ beta-strand) from the NH₂-terminal domain of the FimC chaperone is used to complement the pilin domain by being inserted between the second half of the A strand and the F strand of the domain (Figure 10C). Thus, the final strand (F) of FimH forms a parallel beta-strand interaction --

Examiner's Comment

3. According to a conversation with Mr. Timothy McBride, support for the above amendment on page 72 of the specification can be found in provisional application Serial No. 60/148,280, from which the instant application claims priority and which was incorporated by reference into the instant application, on page 29, line 6, through page 31, line 22, and on page 32, line 1 through page 34, line 21. Support for the above amendment on page 78 of the specification can be found in Figure 11B of provisional application Serial No. 60/148,280, and Figure 10B and page 13, lines 9-13, of the instant specification.

4. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shubo (Joe) Zhou, whose telephone number is 571-272-0724. The examiner can normally be reached Monday-Friday from 8 A.M. to 4 P.M. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ardin Marschel, Ph.D., can be reached on 571-272-0718. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to Patent Analyst Tina Plunkett whose phone number is (571) 272-0549.

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Shubo (Joe) Zhou, Ph.D. 

Patent Examiner

John S. Brusca 29 December 2005
JOHN S. BRUSCA, PH.D.
PRIMARY EXAMINER